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# Barrier properties of spray-dried emulsions containing flavorings or unsaturated triglycerides

Bruna Barbon Paulo [a,*], Izabela Dutra Alvim [b], Gary Reineccius [c], Ana Silvia Prata [a]

[a] Department of Food Engineering, School of Food Engineering, State University of Campinas (UNICAMP), 80 Monteiro Lobato Street, CEP: 13083-862, Campinas, SP, Brazil
[b] Institute of Food Technology (ITAL), Center for Technology of Cereals and Chocolates, 2880 Brasil Avenue, CEP: 13070-178, Campinas, São Paulo, Brazil
[c] Department of Food Science and Nutrition, University of Minnesota, 1334 Eckles Avenue, 55108, St. Paul, Minnesota, USA

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ABSTRACT

Oxygen diffusion through spray dried particles is the crucial factor in primary determination of product shelf-life. This work investigates the main factors influencing the oxygen barrier properties of maltodextrin-based spray-dried emulsions with different surface-active components (SAC). Low amounts (1.2–4.8 g/100 g emulsion) of SAC (whey protein isolate, gelatin or Capsul®) were used to encapsulate an unsaturated triglyceride (fish oil) or a volatile (orange essential oil) at 6 g/100 g emulsion (1:4 oil:wall material). Differences were observed in the oxygen uptake. As the encapsulation efficiency and the bulk matrix were not necessarily related to the oxidation, the interfacial membrane surrounding the oil droplets is suggested to be determinant in the oxidative stability. The protein matrices showed antioxidant capacity that also can contribute to high protection. This work provided insights about the understanding of how barrier properties of powders affect oxidation.

## 1. Introduction

Flavorings and unsaturated triglycerides play an important role as functional substances in food products, even though they are highly susceptible to degradation reactions. Numerous studies have been published on spray drying methods to preserve the sensory and nutritional properties of these fragile components (Carneiro, Tonon, Grosso, & Hubinger, 2013; Ren et al., 2020). However, the fundamental question remains as to the mechanism of how spray-dried particles structure limits oxidation.

The factors reported as responsible for the oxidation of spray-dried compounds are: a) the particle properties influencing the exposure of the outer surface to the oxygen; b) the presence of surface oil and the load of oil in the particles (Reineccius & Yan, 2016; Wang, Adhikari, & Barrow, 2019); c) the diffusion of the oxygen from outside particles and/or from the entrapped air inside the vacuole (Reineccius & Yan, 2016) crossing the barriers (Subramaniam et al., 2013), which is influenced mainly by the thickening and composition of the particle surface (Adhikari, Howes, Bhandari, & Langrish, 2009; Adhikari, Howes, Wood, & Bhandari, 2009), the porosity of the particle, i.e., the free volume in the matrix, and/or the oxygen solubility within the matrices (Drusch et al., 2009; Moreau & Rosenberg, 1998, 1999; Ubbink, 2009; Wang, Adhikari, & Barrow, 2019); d) the presence of antioxidant materials in the formulation (Wang, Adhikari, Mathesh, Yang, & Barrow, 2019; Wang, Adhikari, & Barrow, 2019). All these factors are intrinsically related to the components of the formulation.

Important sources of bioactive compounds (e.g. essential oils or highly unsaturated triglycerides) are generally immiscible in the aqueous phase, thus, natural or modified food-grade biopolymers, such as proteins and/or modified starches, are usually added as surface-active compounds (SAC) to form an emulsion. In the emulsion step, SAC are responsible to form an interfacial layer and increase the stability of emulsions (Francisco, de Oliveira Júnior, Marin, Alvim, & Hubinger, 2020; McClements & Gumus, 2016), and may increase the oil retention and reduce the surface oil during the drying. However, the amount of surface oil may not be necessarily responsible for the oxidative stability provided by spray-dried particles (Drusch & Berg, 2008). Carneiro et al. (2013), for example, found that whey protein isolate as wall materials presented higher oxidative stability of encapsulated flaxseed oil than modified starches, which, conversely, presented lower encapsulation efficiency. Drusch and Berg (2008) showed that the surface oil can even protect other fractions of the extractable oil and that the extractable oil cannot be used to predict shelf-life of microencapsulated oils. Therefore, other factors like oxygen permeability, distribution of the oil and its

* Corresponding author.
*E-mail addresses:* brunabarbonpaulo@gmail.com, brunabpaulo@hotmail.com (B.B. Paulo).

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chemical stabilization become limiting for long-term stability (Drusch et al., 2012).

In the dried matrix, SAC play a fundamental role in the three main barriers present in the spray-dried particle: particle surface (interface between air and wall materials), the bulk phase and the interface between oil and wall materials (Reineccius & Yan, 2016). Some works have shown that protein molecules can migrate to the droplet-air interface, transforming a protein-rich film into a non-sticky glassy state upon drying (Adhikari, Howes, Bhandari, & Langrish, 2009). Ubbink and his research group (Kilburn et al., 2004; Townrow, Kilburn, Alam, & Ubbink, 2007; Townrow, Roussenova, Giardiello, Alam, & Ubbink, 2010) have shown that carbohydrates can change the molecular organization of amorphous carbohydrate matrices. Drusch's research group has shown the reduction of the free intermolecular volume decreased the oxidation rate of spray-dried powders (Drusch et al., 2009, 2012; Serfert et al., 2013, 2013). Other works affirm that when low-molecular weight carbohydrates (sucrose, glucose, maltose, etc.) are blended with high-molecular weight carbohydrates (maltodextrin and emulsifying agents - gum acacia or modified starches), they can plug the intermolecular voids in the molecular chains (Reineccius & Yan, 2016), reducing the oxygen diffusion (Charve & Reineccius, 2009; Moreau & Rosenberg, 1998, 1999; Ubbink, 2009, 2018). However, to our knowledge, there is no study in the literature discussing how small amounts of different types of SAC can influence the main mechanisms and factors that limit the oxidation of spray-dried particles.

Based on recent scientific findings, this research is an effort to understand the influence of the type of SAC on the oxygen barrier properties of spray dried particles containing flavorings and unsaturated triglycerides. Gaining this understanding would potentially permit the industry to engineer new encapsulation matrices that provide a better oxygen barrier and may reduce particle materials costs.

## 2. Material and methods

### 2.1. Material

An unsaturated oil, represented by fish oil (FO) 33:22 (EPA:DHA) ($\rho$ = 0.875 g/mL, $\mu$ = 4.6 mPa s, $\sigma$ = 32.3 mN/m at 25 °C, Incromega E3322-LQ-(LK), Croda Europe Ltda., UK), or a flavoring, represented by the orange essential oil (OEO) ($\rho$ = 0.843 g/mL, $\mu$ = 1.1 mPa s, $\sigma$ = 28.4 mN/m at 25 °C, Citrosuco S/A Agroindustria, Brazil) were used as core materials for spray drying. The aqueous phase was composed of maltodextrin (MD) (MOR-REX® 1910, 10 DE, Ingredion Brasil Ingredientes Ltda, Mogi Guaçu, Brazil), and by SAC: Octenyl succinic anhydride (OSA)-modified starch (MS) (Capsul®, Ingredion Brasil Ingredientes Ltda, Mogi Guaçu, Brazil) or proteins: gelatin (GE) (100 H 30, Rousselot Gelatinas do Brasil Ltda, Amparo, Brazil) or whey protein isolate (WPI) (Fonterra Ltda, Tokoroa, New Zealand).

### 2.2. Production of particles

Based on previous work on emulsion stability by Paulo, Alvim, Reineccius, & Prata (2020), emulsions containing WPI, GE or MS as SAC were chosen for study. Each SAC was combined with MD at the concentrations chosen (Table 1). FO or OEO (6 g oil/100 g emulsion) was dispersed in the aqueous wall material solutions (24 g wall materials/100 g emulsion). The emulsions (30 g total solids (carrier solids plus active material)/100 g emulsion) were produced using the Ultra-Turrax mixer (T18BS1, IKA T18 basic, Germany) at 11,200 rpm for 90 s. The resultant emulsions and the aqueous wall material solutions (without oil) were spray dried (B-290, Büchi, Flawil, Switzerland) with a two-fluid atomizer (D.I. 0.7 mm) at a feed rate of 0.8 L/h. The experimental tests were performed with inlet and outlet air drying temperature of 180 °C and 90 ± 3 °C, respectively. The ratio of the oil to wall material was maintained at 1:4 (w/w), which means that the final composition of all particles was 20% oil and 80% of the wall material (dry basis) (Charve & Reineccius, 2009; Jafari, Assadpoor, Bhandari, & He, 2008; Jafari, Assadpoor, He, & Bhandari, 2008).

### 2.3. Encapsulation efficiency

The encapsulation efficiency (EE) for both oils was determined as the ratio between the oil encapsulated in the matrix (total oil (TO) – surface oil (SO)), and the oil in or on the surface of the capsules (TO), as presented in Equation (1).

$$\mathbf{EE} = \frac{\mathbf{TO} - \mathbf{SO}}{\mathbf{TO}} \times 100 \quad (1)$$

#### 2.3.1. FO-particles

The SO was measured according to Carneiro et al. (2013) and the TO was determined according to Bligh and Dyer (1959).

#### 2.3.2. OEO-particles

The SO was determined according Ramos, Silveira Júnior, and Prata (2019) and the TO was determined using Clevenger distillation method according to Jafari, He, and Bhandari (2007).

For both oils, the retention of the samples was also calculated, which is the proportion of the oil remaining in the powder. It is calculated as follows Equation (2).

$$\text{Retention } (\%) = \frac{\text{TO}}{\text{Theoretical oil (initial TO)}} \times 100 \quad (2)$$

### 2.4. Moisture content and water activity

The moisture content of the spray-dried powders was determined in triplicate by the Karl Fischer titration method using a Karl Fischer Titrando 901 (Metrohm Ltd., Switzerland). Between 0.2 and 0.3 g of the powder samples were added to a mixture of methanol and formamide (2:1), and then titrated with standardized Karl Fischer reagent. The water activity ($a_w$) was measured using a water activity meter (AquaLab 4TEV, Decagon Devices Inc., Pullman, USA) at 25.00 ± 0.50 °C.

### 2.5. Mean particle diameter and size distribution

The mean diameter and the size distributions of the powders were obtained via laser diffraction method using an LV 950-V2 equipment (Horiba, Kyoto, Japan) by wet method, with dispersion in 99.5%

**Table 1**
Composition of FO and OEO-emulsions (g/100 g emulsion).

| Sample | Oil (6 g/100 g emulsion) | Wall materials (24 g/100 g emulsion) | | | | |
|---|---|---|---|---|---|---|
| | | MD (g/100 g emulsion) | MS (g/100 g emulsion) | WPI (g/100 g emulsion) | GE (g/100 g emulsion) | SAC:Oil |
| FO_MD+MS | FO | 22.8 | 1.2 | – | – | 1:5 |
| FO_MD+WPI | FO | 22.8 | – | 1.2 | – | 1:5 |
| FO_MD+GE | FO | 20.4 | – | – | 3.6 | 3:5 |
| OEO_MD+MS | OEO | 20.4 | 3.6 | – | – | 3:5 |
| OEO_MD+WPI | OEO | 22.8 | – | 1.2 | – | 1:5 |
| OEO_MD+GE | OEO | 19.2 | – | – | 4.8 | 4:5 |

ethanol.

### 2.6. Bulk density

Approximately 2 g of the powder samples were added in a 10 mL graduated cylinder and gently compacted by tapping it on the countertop 5 times. The bulk density ($\rho_{bulk}$) was calculated as the ratio of the powder mass to the volume occupied in the cylinder.

### 2.7. Apparent density

A 50 mL pycnometer was filled with 99.5% ethanol, and the corresponding mass was determined ($m_{liquid}$). Then, an amount of particles ($m_{particles}$) was added to the empty pycnometer, the volume was completed with ethanol, and the new mass of the liquid-particle system ($m_{LP}$) was quantified. The apparent density of the particle was calculated by Equation (3).

$$\rho_{ap} = \frac{\rho_{liquid} \times m_{particles}}{\left[m_{particles} - \left(m_{LP} - m_{liquid}\right)\right]} \quad (3)$$

### 2.8. Solubility

The solubility of the particles was evaluated according to the method proposed by Fernandes et al. (2016).

### 2.9. Hygroscopicity

The hygroscopicity was determined according to the method followed by Tonon, Brabet, and Hubinger (2008).

### 2.10. Morphology

This characterization was performed using a scanning electron microscope (DSM 940A FOCUS, Zeiss, Jena, Germany). The samples were fixed on metal surfaces and coated with gold. The observations were made using electronic image capture with 500 × and 1000 × magnification.

### 2.11. Determination of the free volume of the particles

#### 2.11.1. Particle density

Particle density of the powder was determined using a helium pycnometer (AccuPyc 1330, Norcross, USA).

#### 2.11.2. Positron annihilation lifetime spectroscopy (PALS)

Based on the methodology of Drusch et al. (2009), the samples of spray-dried carrier matrix without oil were equilibrated at 25 °C and 33% RH under a nitrogen environment prior to analysis. Firstly, tablets of approximately 300 mg of samples were produced by using a tableting die (8 mm diameter) and a Carver press for 10 s at 500 psi. For performing PALS, a $^{22}$Na (radioactive source) film was placed between two powder tablets (replicates). Two sensors on each side of these replicates along with the $^{22}$Na film were put for detecting the gamma ray energies ($^{22}$Na degradation). These two gamma ray detectors detect the energy emitted from the radioactive decay of $^{22}$Na (the start gamma ray) and the energy emitted by the annihilation of the created positron with an electron, which is contained in the sample (the annihilation gamma ray). The time spent between these two energy detections was calculated and it corresponds to the lifetime of a positron. The positron annihilation spectra of each sample were recorded and analyzed for approximately 1 h. The results were expressed as the size of the intermolecular hole. Security measures associated with handling radioactive compounds were taken into account.

### 2.12. Determination of oxidation

The oxygen consumption was determined using an Oxipres apparatus (Mikrolab Aarhus A/S, Højbjerg, Denmark). Twenty g of powder or bulk (unencapsulated) oil was placed inside a hermetically closed iron vessel, and the system was maintained at high oxygen pressure (5 bar) and temperature of 60 °C for 48 h. The oxidation of the bioactives requires oxygen consumption and, therefore, pressure reduction. The results given by the instrument are expressed as pressure difference ($\Delta P$) which was converted to the volume of consumed oxygen, as follows in Equations (4) and (5).

$$\Delta n = \frac{\Delta P\, V}{R\, T} \quad (4)$$

$$V_{O2} = \frac{\Delta n\, MM}{\rho_{O2}\, mass_{powder}} \quad (5)$$

Where $\Delta n$ is the variation of the number of moles (consumed $O_2$ mol), $\Delta P$ is the pressure difference (N/m$^2$), R is the gas constant (8.314 J/mol K), T is the temperature (333 K) and V is the free volume in the iron vessel, which is given by the difference between the total volume (125 mL) and the volume occupied by the sample (49.09 mL for oil, and for particle bed $V_{occupied} = mass_{powder}/\rho_{bulk}$), $V_{O2}$ is the consumed $O_2$ volume (mL), MM is the $O_2$ molar mass (32 g/mol), $\rho_{O2}$ is the $O_2$ density (0.00133 g/mL), $mass_{powder}$ is the amount of sample placed in the equipment (20 g).

### 2.13. Antioxidant activity for wall materials

The protein solutions at a range of concentration tested in the previous study of emulsion stability (Paulo, Dutra, Reineccius, & Prata, 2020) (0.48 and 6 g/100 g emulsion) were evaluated in relation to the antioxidant activity by Ferric reducing antioxidant power (FRAP) and Ability to Scavenge ABTS free radical (ABTS) according the methodology of Rufino et al. (2006, 2007), whose the spectrophotometric absorbance was read at 595 nm and 734 nm (T60 PG Instruments, United Kingdom), respectively. Antioxidant capacity was then calculated according to a calibration curve using aqueous solutions of Trolox (100–1000 μM). The results were expressed as Trolox equivalents per g of sample (mM TE/g).

## 3. Results and discussion

The oxidative stability of the FO and OEO-containing powders is shown in Fig. 1 and Table 2.

In a general view for comparing the performance of different SAC, MS-powders were the most susceptible to oxidation for both oils, observed by the greatest pressure drop and, therefore, the highest oxygen consumption. These powders presented the shortest induction time for FO containing-powders (17 h), but for OEO containing-particles it could not be detected (Fig. 1).

The main factors influencing the oxidation of two different encapsulated oils (orange essential oil or fish oil) and the effectiveness of the type of SAC to protect them against oxidation are discussed in the next topics, which are divided based on the fundamental factors that would impact the oxidation reaction.

### 3.1. Particle properties

The physicochemical properties of the powders are summarized as a function of the formulation composition in Table 2. All these parameters are related to the particle properties, and, therefore, to the exposure of the outer surface to the oxygen.

Similar bulk ($\rho_{bulk}$ ~ 0.4 g/mL) and apparent densities ($\rho_{ap}$ ~1.5 g/mL and $\rho_{ap}$ ~1.0 g/mL for FO and OEO containing-powders, respectively), mean size (10–13 and 8–11 μm for FO and OEO-powders,

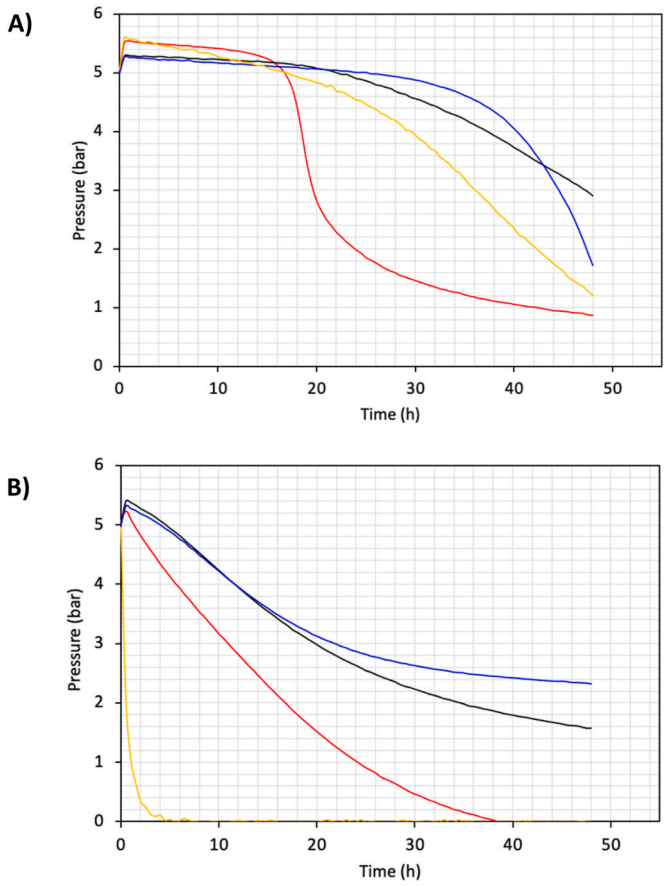


**Fig. 1.** Accelerated oxidative stability test using Oxipres at 60 °C and 5 bar for the bulk oil (—) and the spray-drying powders containing the wall materials MS+MD (—), WPI+MD (—), and GE+MD (—) and the active components: A) Fish Oil; B) Orange Essential Oil. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

respectively), and monomodal size distribution (Fig. 2) were observed for all loaded particles. The scanning electron microscopy (SEM) images of all powders (Fig. 3) show similar morphological characteristics, i.e., a spherical shape and shriveled surface without apparent fissures or cracks in the structure. All powders presented similar solubility in water (~65%), except MS-particles containing OEO (~55%). The highest span observed for GE-powders is the result of the highest viscosity of the GE-emulsions, as reported previously by Paulo et al. (2020).

Also, it is well known that the high moisture content and water activity can accelerate the degradation reactions, including diffusion of the oxygen (Anwar & Kunz, 2011; Nelson & Labuza, 1992). Although some powders presented significant differences in the water activity and moisture content, all spray-dried microparticles had a dry matter content of at least 95%, similar hygroscopicity (~8%) and an $a_w \cong 0.2$, which is in the range of the lowest lipid oxidation and the long-term stability against microbiological spoilage (Nelson & Labuza, 1992; Soottitantawat et al., 2004).

The similar physicochemical properties are expected, because the same operating conditions were maintained for all formulations (Anantharamkrishnan & Reineccius, 2018; Reineccius & Yan, 2016). Although the SAC composition was different, ranging from 4 to 16% (w/w dry particle), it is still small compared to the maltodextrin concentration, which is the major component of all samples (>64% w/w dried particle). Therefore, the differences observed in the oxidation reaction may be related to the barrier structures and/or oil entrapment obtained for each formulation.

### 3.2. Encapsulation efficiency

Analyzing how EE can affect the oxidation based on oxygen consumption (Fig. 1) and the values of total volume of $O_2$ consumed (mL/g total oil) (Table 2), the oil type is a dominant factor in oxidation.

In this context, an important difference between the oils should be highlighted. For flavor encapsulation, there is a potential loss of SO due to evaporation, but this does not occur for unsaturated triglycerides. Thus, the SO present in FO loaded powders may accelerate the oxidation (Drusch et al., 2012; Reineccius & Yan, 2016), as shown in a Drusch's study for particles containing fish oil as active and gum arabic in combination with octenylsuccinate-derivatized starch as encapsulating matrix (Drusch, 2007). The results of oil entrapment in the particles are shown in Table 2 and they confirm these differences.

OEO-powders presented lower SO (0.8% w/w) than FO-powders (SO > 5%) and the TO was higher even for the same formulation of wall material, e.g. WPI+MD-powders (85% and 71% for OEO and FO, respectively). In general, the OEO-powders presented a higher oxygen consumption even at 24 h (Table 2) than FO-powders without an induction period (auto-protection of the oil) (Fig. 1), which could be related to the higher amount of TO. In the literature, Shen, Augustin, Sanguansri, and Cheng (2010) also found higher oxygen uptake in powders containing the higher oil content at the end of the induction period. Moreover, other reason for OEO-powders presenting higher susceptibility to oxidation than FO-powders is the different physicochemical structure between the volatile oil (orange essential oil) and the unsaturated triglyceride (fish oil). It is expected the small and more hydrophilic molecules of the OEO-droplets have higher vapor pressure than the long-chain fatty acids present in the FO-droplets, being, therefore, more volatile. After drying, the OEO-molecules may have been released easily and faster from the matrix, mainly under higher pressure and temperature during the test of accelerate shelf-life. As consequence, they consumed more oxygen than the FO-powders.

The TO also can explain the results of GE containing-powders, which presented one of the lowest oxygen consumptions (83 and 68 mL $O_2$/g oil for FO and OEO-powders, respectively), but the highest SO (~8% and 0.83% for FO and OEO-powders, respectively), confirming the oxidative stability is not necessarily related to the SO. The low EE of GE-powders (42.5% and 94.6% for FO and OEO-powders, respectively) and lowest oil retention (~69% for both oils) may be explained for the mechanism of emulsion stability, which was more related to the high viscosity than the reduction of the interfacial tension (Paulo et al., 2020). Gómez-Mascaraque and López-Rubio (2016) also observed that gelatin yielded lower values of encapsulation efficiency than globular proteins (WPC and soy protein isolate) using electrospraying process.

MS and WPI containing-emulsions presented similar emulsion properties (Paulo et al., 2020), but different oxidative stability. MS containing-particles presented the higher oxygen uptake (127 and 94 mL $O_2$/g oil for FO and OEO-particles, respectively) than WPI containing-particles (56 and 71 mL $O_2$/g oil for FO and OEO-particles, respectively). This result has also been reported in the literature, e.g., Charve and Reineccius (2009) showed proteins as better wall materials for limiting limonene oxidation than Capsul®.

However, it is expected that modified starches (Hi Cap® and/or Capsul®) lead to higher encapsulation efficiencies than whey protein concentrates, regardless of the oil type (Carneiro et al., 2013; Charve & Reineccius, 2009; Jafari, Assadpoor, Bhandari, & He, 2008). In our study, the differences in the EE between MS and WPI were not significant for both oils, as result of the similar emulsion properties (Paulo et al., 2020), but they can give some insights about the oxidation. For OEO-particles, the oil retained (load) was slightly higher for MS+MD (87.6%) than WPI+MD (85%), suggesting this as a potential factor for oxidation for flavor powders. For FO-particles, MS containing-powders presented lower oil retention (64%) and higher SO (6.7%) compared to WPI+MD (oil retention = 70.6%, SO = 5.5%) and these differences were not necessarily related, at the same proportion, to the oxidative

**Table 2**
Physicochemical properties of the fish oil (FO) and orange essential oil (OEO) particles.

| Physicochemical properties | FO_MS+MD | FO_WPI+MD | FO_GE+MD | OEO_MS+MD | OEO_WPI+MD | OEO_GE+MD |
|---|---|---|---|---|---|---|
| Total oil (g oil/100 g dry powder) | 12.82 ± 0.78$^{a}$ | 14.13 ± 0.72$^{a}$ | 13.81 ± 0.34$^{a}$ | 17.53 ± 0.53$^{A}$ | 16.99 ± 2.68$^{AB}$ | 13.81 ± 0.51$^{B}$ |
| Oil retention (g total oil/g theoretical oil) | 64.11 ± 3.91$^{a}$ | 70.64 ± 3.58$^{a}$ | 69.04 ± 1.68$^{a}$ | 87.63 ± 2.65$^{A}$ | 84.97 ± 13.42$^{AB}$ | 69.03 ± 2.53$^{B}$ |
| Surface oil (g oil/100 g dry powder) | 6.73 ± 1.67$^{ab}$ | 5.46 ± 0.59$^{b}$ | 8.10 ± 0.82$^{a}$ | 0.72 ± 0.08$^{A}$ | 0.78 ± 0.08$^{A}$ | 0.83 ± 0.03$^{A}$ |
| Encapsulation Efficiency (%) | 47.93 ± 9.56$^{b}$ | 61.32 ± 3.81$^{a}$ | 42.50 ± 3.66$^{b}$ | 95.89 ± 0.43$^{A}$ | 95.44 ± 0.45$^{AB}$ | 94.59 ± 0.18$^{B}$ |
| Hygroscopicity (%) | 8.38 ± 1.69$^{a}$ | 8.15 ± 0.40$^{a}$ | 7.95 ± 0.13$^{a}$ | 8.27 ± 0.88$^{A}$ | 8.21 ± 0.08$^{A}$ | 7.29 ± 0.19$^{A}$ |
| Moisture (% w/w) | 4.79 ± 0.61$^{a}$ | 4.61 ± 0.42$^{a}$ | 4.98 ± 0.32$^{a}$ | 5.38 ± 0.16$^{AB}$ | 5.75 ± 0.34$^{A}$ | 4.95 ± 0.48$^{B}$ |
| Water activity | 0.23 ± 0.02$^{b}$ | 0.18 ± 0.03$^{c}$ | 0.25 ± 0.01$^{a}$ | 0.18 ± 0.01$^{B}$ | 0.18 ± 0.01$^{B}$ | 0.24 ± 0.02$^{A}$ |
| Solubility in water (%) | 65.52 ± 0.53$^{a}$ | 65.20 ± 2.38$^{a}$ | 67.11 ± 5.63$^{a}$ | 54.95 ± 2.41$^{B}$ | 64.93 ± 2.16$^{A}$ | 67.08 ± 0.38$^{A}$ |
| Bulk density (g/mL) | 0.443 ± 0.026$^{a}$ | 0.413 ± 0.001$^{a}$ | 0.394 ± 0.020$^{a}$ | 0.382 ± 0.018$^{A}$ | 0.384 ± 0.005$^{A}$ | 0.374 ± 0.039$^{A}$ |
| Apparent density (g/mL) | 1.50 ± 0.07$^{a}$ | 1.34 ± 0.27$^{a}$ | 1.67 ± 0.46$^{a}$ | 1.06 ± 0.12$^{A}$ | 1.09 ± 0.16$^{A}$ | 1.01 ± 0.19$^{A}$ |
| Oil droplet diameter ($D_{3,2}$) (μm)[a] | 1.85 ± 0.17 | 2.18 ± 0.02 | 10.79 ± 0.14 | 2.42 ± 0.27 | 2.41 ± 0.00 | 9.00 ± 0.98 |
| Particle volume mean diameter ($D_{4,3}$) (μm) | 11.99 ± 1.43$^{a}$ | 9.65 ± 1.95$^{a}$ | 13.23 ± 2.10$^{a}$ | 8.38 ± 0.57$^{A}$ | 8.23 ± 0.59$^{A}$ | 10.63 ± 1.91$^{A}$ |
| Particle - $D_{10}$ (μm) | 2.98 ± 0.44$^{a}$ | 2.94 ± 0.33$^{a}$ | 2.55 ± 0.52$^{a}$ | 3.81 ± 0.03$^{A}$ | 3.04 ± 0.30$^{B}$ | 2.00 ± 0.18$^{C}$ |
| Particle - $D_{50}$ (μm) | 10.41 ± 2.04$^{a}$ | 7.49 ± 0.31$^{b}$ | 7.77 ± 0.18$^{b}$ | 7.68 ± 0.50$^{A}$ | 6.60 ± 0.18$^{B}$ | 6.73 ± 0.50$^{B}$ |
| Particle - $D_{90}$ (μm) | 19.84 ± 1.46$^{ab}$ | 17.04 ± 3.47$^{b}$ | 28.57 ± 6.90$^{a}$ | 13.76 ± 1.20$^{B}$ | 12.52 ± 0.80$^{B}$ | 22.28 ± 4.50$^{A}$ |
| Particle - Span | 1.66 ± 0.30$^{b}$ | 1.87 ± 0.42$^{b}$ | 3.35 ± 0.95$^{a}$ | 1.29 ± 0.07$^{B}$ | 1.44 ± 0.17$^{B}$ | 3.00 ± 0.57$^{A}$ |
| Ratio Oil droplet size ($D_{3,2}$): Particle size ($D_{4,3}$) | 1:6 | 1:5 | 11:13 | 1:4 | 1:4 | 9:11 |
| Average ($\tau_{o\text{-}Ps}$) of the o-Ps lifetime (ns) | 1.383 | 1.441 | 1.378 | 1.404 | 1.441 | 1.370 |
| Pore size (nm) | 0.220 | 0.227 | 0.219 | 0.222 | 0.227 | 0.218 |
| Particle density (g/mL) | 1.321 ± 0.006$^{a}$ | 1.311 ± 0.014$^{b}$ | 1.246 ± 0.014$^{c}$ | 1.217 ± 0.001$^{B}$ | 1.323 ± 0.012$^{A}$ | 1.200 ± 0.011$^{B}$ |
| Oxygen consumption (mL $O_2$/g total oil) after 24 h | 96.50 | 9.03 | 5.93 | 75.90 | 51.70 | 55.23 |
| Oxygen consumption (mL $O_2$/g total oil) after 48 h | 126.57 | 56.01 | 82.77 | 94.32 | 71.09 | 67.60 |

Data are expressed as means ± standard deviations. Different lowercase and capital letters represent a significant difference ($p < 0.05$) for FO and OEO particles, respectively (Duncan's test).

[a] Data obtained from Paulo et al. (2020).

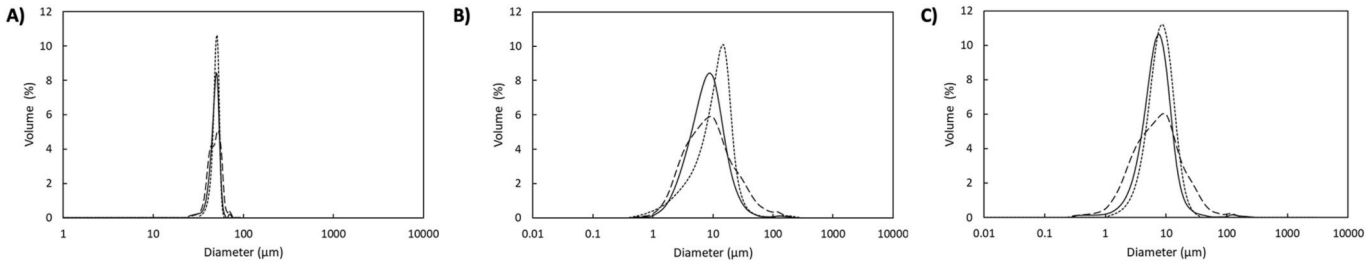


**Fig. 2.** Particle size distribution diagrams of spray-dried particles containing the wall materials: MS+MD (▬▬▬), WPI+MD (▬▬▬), GE+MD (▬ ▬ ▬): A) atomized wall materials without oil; B) FO-particles; C) OEO-particles.

stability. For instance, at 24 h, MS-particles consumed a significantly higher amount of oxygen (97 mL $O_2$/g total oil) than WPI-powders (9 mL $O_2$/g total oil).

In addition, if the rate of oxygen consumption was due to the SO content, it is logical to expect a change in the slope of the lines for those higher SO powders after a certain time due to the initial rapid oxidation of the SO followed by a slower oxidation of the encapsulated oil (Anandaraman & Reineccius, 1986). At the same perspective, even though GE-particles had presented higher SO than WPI-powders for both FO and OEO, they presented similar behavior of oxygen consumption to the WPI-particles (Fig. 1). Similarly to our study, Carneiro et al. (2013) found higher oxidative stability of flaxseed oil encapsulated in matrices containing maltodextrin and whey protein isolate, which, conversely, presented higher SO.

Therefore, in this study, the higher SO was not responsible for lower oxidation stability, showing other factors are also controlling the rate of oxidation.

### 3.3. Diffusion of the oxygen through the barriers of the particle

During the drying process, there is a formation of the crust at the particle surface and also the bulk matrix. Both barrier structures are important for evaluating the oxygen diffusion. Studies have reported that protein molecules can migrate to the droplet-air interface, transforming a protein-rich film into a non-sticky glassy state upon drying (Adhikari, Howes, Bhandari, & Langrish, 2009). This can explain the efficient barrier provided by the WPI-powders in this study, even with high amounts of TO. Also, WPI presents faster drying kinetics than maltodextrin, when in small amounts (Prata, Garcia, Tonon, & Hubinger, 2013), and, by having a lower molecular-weight compared to modified starch, it may even facilitate the crust formation and avoid the surface oil or loss of oil during the process (Aghbashlo, Mobli, Madadlou, & Rafiee, 2013; Fang & Bhandari, 2012; Rosenberg & Sheu, 1996).

The free volume and the pore size can provide information about the molecular organization of the components, and, therefore, about the oxygen permeability to the microparticles. The entrapped air in the matrix can be represented by the absolute density measurements (Baisier & Reineccius, 1989). The higher particle density indicates a lower air inclusion within the spray-dried particles, which is more protective against oxidation (Drusch, Serfert, Scampicchio, Schmidt-Hansberg, & Schwarz, 2007). In this study, particle density was approximately 1.3 g/mL for all powders, except for FO_MD+GE-particles (1.25 g/mL), showing the amount of air entrapped is not sufficient to influence the oxidative stability, as showed by Baisier and Reineccius (1989).

The average hole size was determined for all samples with respect to o-Ps lifetime τ3 (τo-Ps). For measurements of PALS, an increase of o-Ps life-time is associated with an increase in the size of free volume

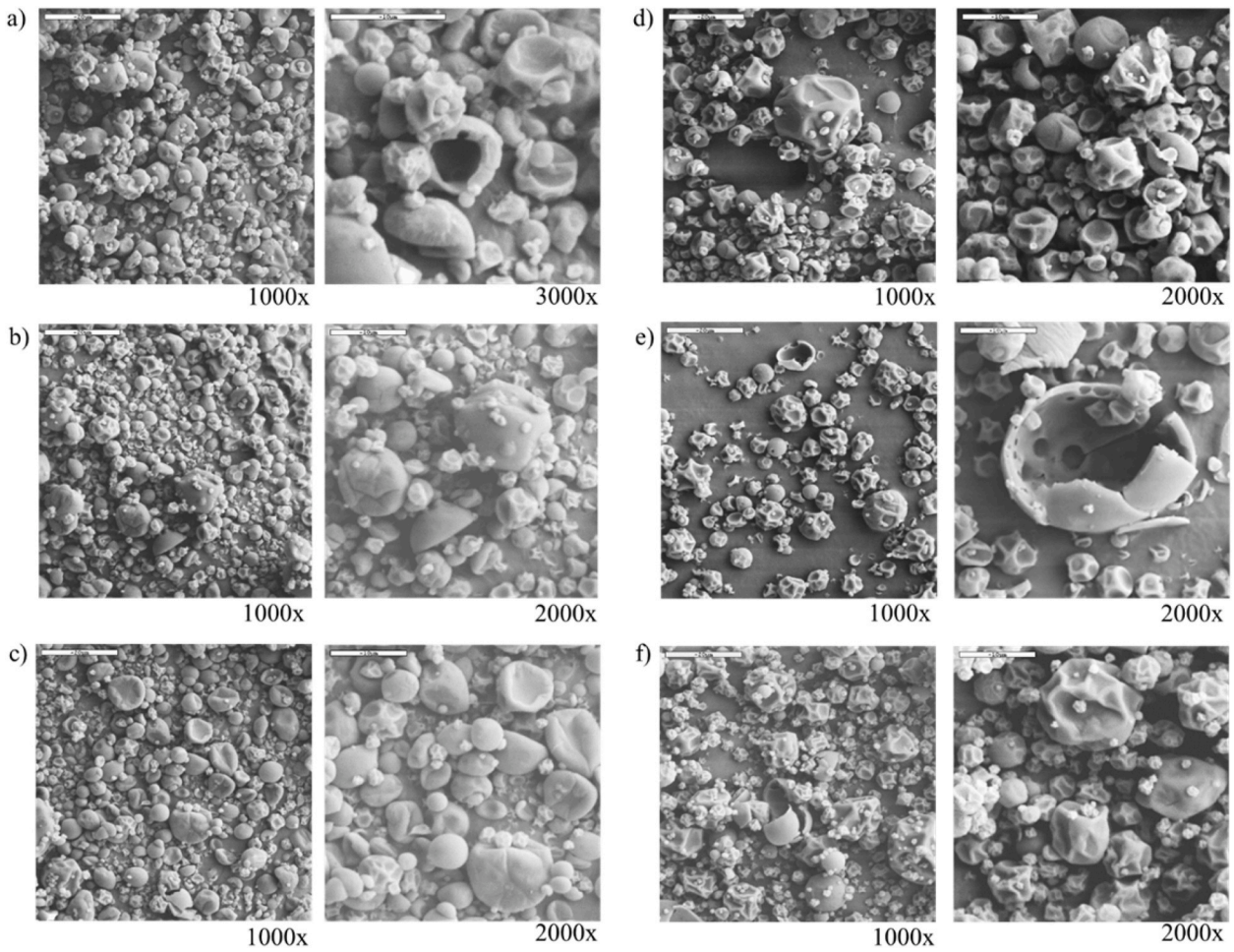


**Fig. 3.** Morphology of the spray-dried particles produced from different wall material combinations: a) FO_MS+MSD; b) FO_WPI+MD; c) FO_GE+MD; d) OEO_MS+MD; e) OEO_WPI+MD; f) OEO_GE+MD.

elements, which may facilitate oxidation of the encapsulated core material (Drusch et al., 2009; Ubbink, 2009, 2016, 2018). The average (τo-Ps) of the o-Ps lifetime and the pore size were in a similar range for all samples (1.4 ns and 0.2 nm) and they are in agreement with the literature (Drusch et al., 2009, 2012).

Therefore, the slight differences in the average hole size and the air inclusion in the matrix were not enough to explain the oxygen uptake through the protective barrier, regardless of the type of oil. This confirms the fact that oxygen permeation across the particle surface and bulk phase takes place not only due to the presence of the pores through which the diffusion occurs, but other factors are also present, as, for example, the solubility of oxygen component through the interfacial membrane surrounding the oil droplets. Drusch et al. (2009) also raised the oxygen solubility as a possible reason for the differences observed in the course of lipid oxidation for fish oil encapsulated by Maltodextrin (18 DE) and Maltodextrin (18 DE) blended with Glucose, which showed a similar τo-Ps and pore size though.

### 3.4. Antioxidant capacity

The lower oxygen uptake of the protein-containing powders may also be attributed to the antioxidant capacity of the powders, as shown by Wang et al., 2019, 2019. Fig. 4 shows the results of antioxidant capacity by FRAP and ABTS assays for protein solutions, since maltodextrins and modified starch are not a class of carbohydrates with potent antioxidant (Wang, Hu, Nie, Yu, & Xie, 2016). Both proteins exhibited potent antioxidant capacity as concentration was increased. Specifically, gelatin solution showed higher antioxidant activity than WPI solution. The FO and OEO-powders had a higher concentration of gelatin than WPI, which can support the result of oxygen stability.

In the literature some studies showed that spray-dried formulations containing whey protein were not oxidized, but were permeable to nitrogen, which has approximately the same molecular size as oxygen. This result demonstrates formation of a dense protein-rich film in the interface oil-water, acting as an efficient oxygen barrier (Moreau & Rosenberg, 1996, 1998, 1999). Therefore, we can conclude, hypothetically, that proteins have antioxidant capacity, and they can migrate to the particle phase interface. They may also accumulate at the emulsion interfaces and form multiple layers through hydrophobic interactions, producing a non-homogeneous distribution of the free volume elements throughout the particle, contributing to a high protection (Drusch et al., 2012). Conversely, Capsul® resulted in powders with similar hole size and higher particle density, but the high-weight carbohydrate molecules resulted in higher oxidation through the bulk carrier matrix and thus facilitated lipid oxidation.

## 4. Conclusions

FO and OEO containing-emulsions were spray-dried using matrices with MD and low amounts of MS, WPI and GE. As the operating conditions were kept the same, and the MD is the major component, all powders presented similar particle properties. However, differences were observed in the oxidation reaction, which may be related to the EE and barrier structures obtained. MS-particles presented the highest

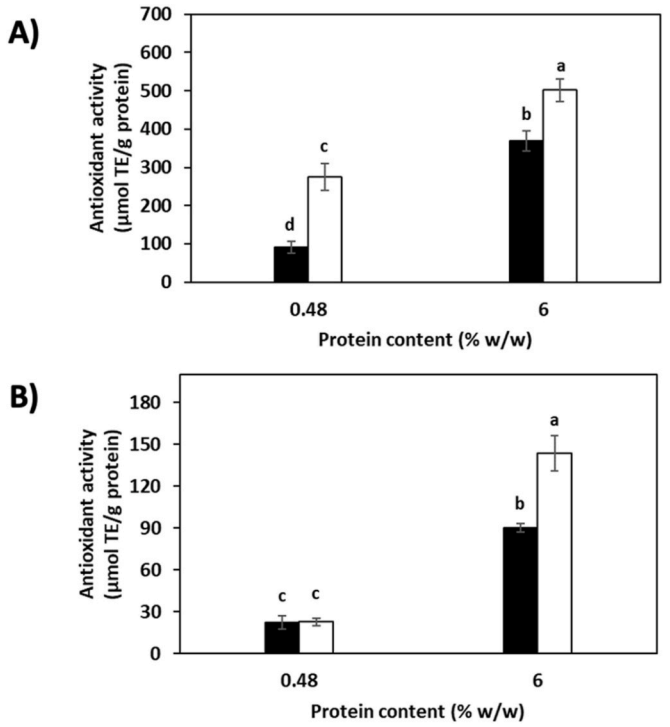


**Fig. 4.** Antioxidant activity of the protein solutions (■WPI; □GE) by means of: a) ABTS, b) FRAP assays.
Different letters in each chart represent statistically significant differences ($p < 0.05$) for the samples (Duncan's test).

oxygen uptake and an EE of ~48% for FO-powders and ~96% for OEO-powders, whereas GE-matrix had presented lower consumption of oxygen and lower EE. This shows lower amounts of TO can result in low consumption of oxygen, but SO is not necessarily related to the oxidative stability. Evaluating the matrix porosity, all powders presented similar particle density, average of the o-Ps lifetime and the pore size as well. Thus, although the presence of the pores in the bulk matrix can allow the oxygen permeation across a protective barrier, these parameters were not enough to explain the oxygen uptake of powders in this study. Therefore, the interfacial membrane surrounding the oil droplets is also crucial for determining the product shelf-life. Besides, GE and WPI-particles presented increased antioxidant effect, which can explain the higher oxidative stability of protein powders.

## CRediT authorship contribution statement

**Bruna Barbon Paulo:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Izabela Dutra Alvim:** Conceptualization, Methodology, Investigation, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Gary Reineccius:** Conceptualization, Resources, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition. **Ana Silvia Prata:** Conceptualization, Methodology, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

## Declaration of competing interest

None.

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